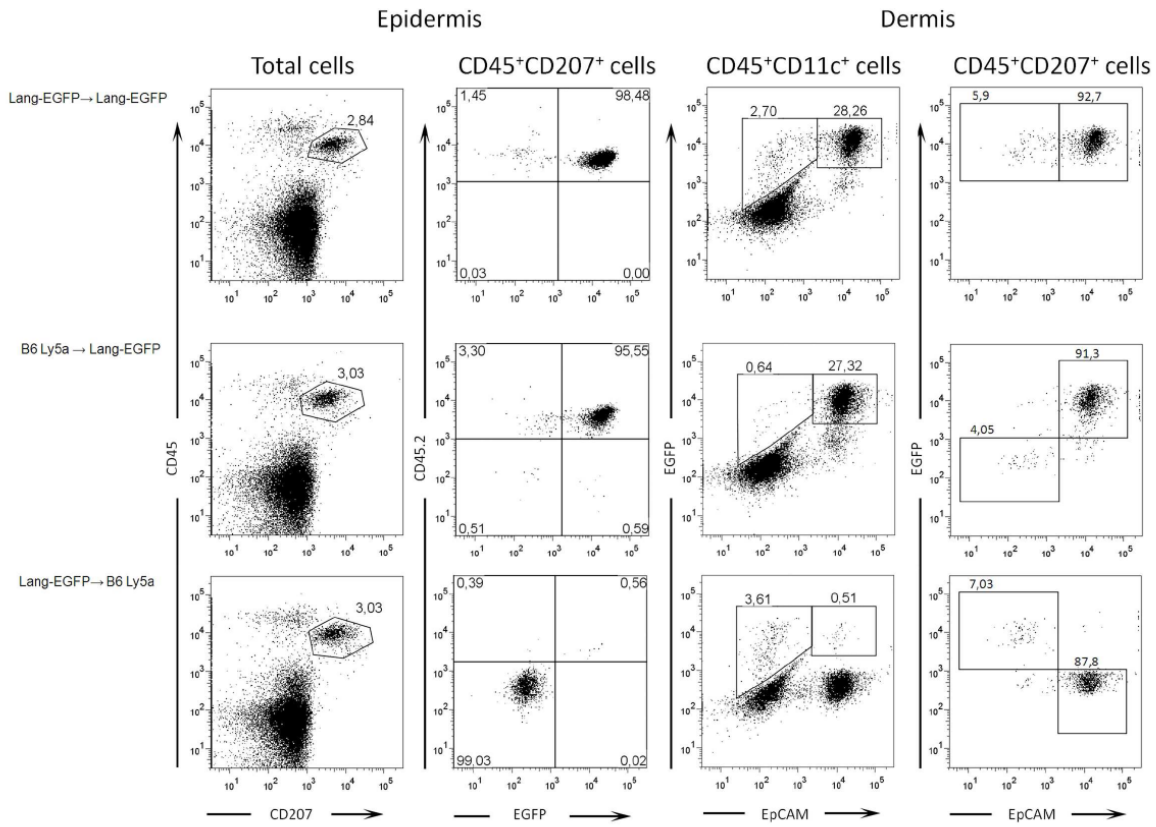
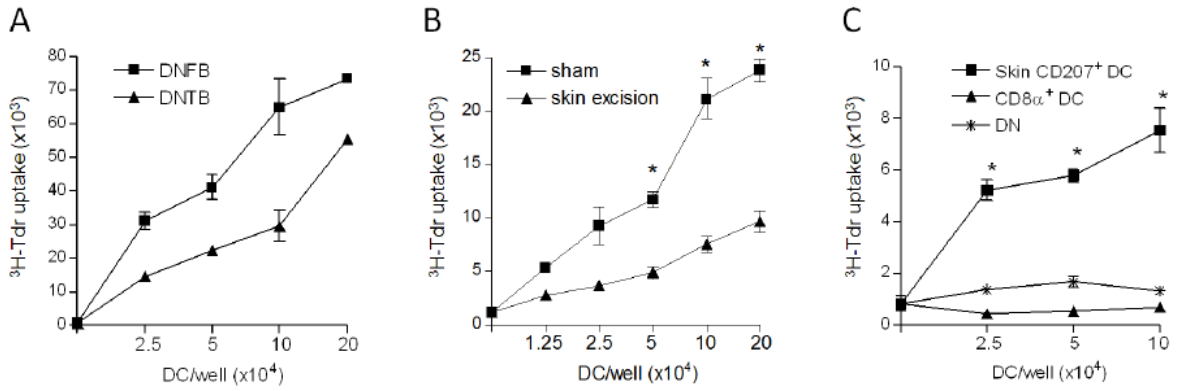
SUPPLEMENTAL FIGURES



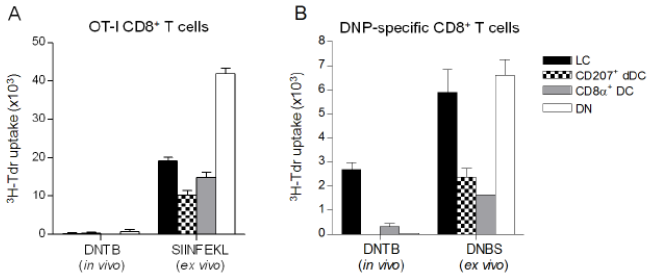
Supplemental Figure 1

Identity of EGFP$^+$ skin DC in BM chimeric mice. 8 weeks after generation of BM chimeras, several mice were sacrificed and epidermal and dermal cell suspensions were prepared by enzymatic digestion of ear skin and were analyzed by flow cytometry for the expression of pan-CD45, CD11c, EGFP, CD207, EpCAM and CD45.2. In B6 ly5a (CD45.1)→Lang-EGFP(CD45.2) chimeric mice, the vast majority of EGFP$^+$ cells present in the epidermis and dermis were host-derived (CD45.2$^+$) LC (CD207$^+$EpCAM$^+$) and CD207$^+$ dDC were EGFP$^-$. By contrast, the few EGFP$^+$ DC present in Lang-EGFP→ B6 ly5a mice were only detected in the dermis and corresponded to CD207$^+$ dDC (EpCAM$^-$), while all LC present in epidermis and dermis were EGFP$^-$.



Supplemental Figure 2

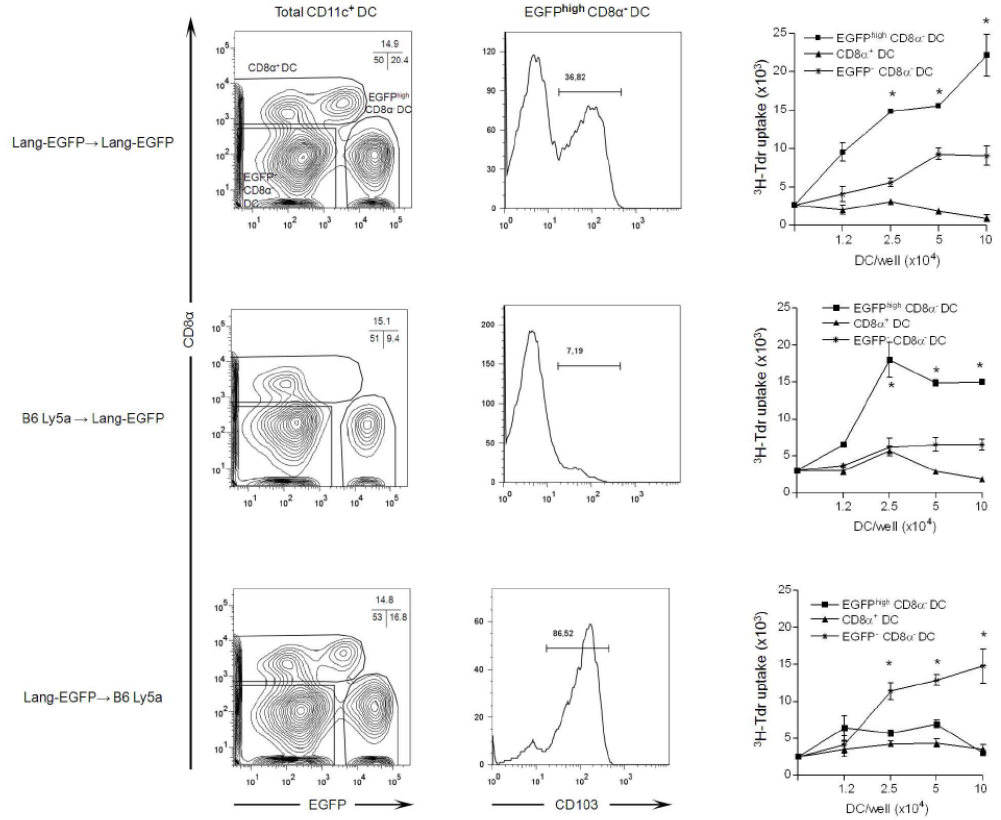
Presentation of haptenated-Ag to CD8$^+$ T cells after skin exposure to DNTB is mostly restricted to skin-derived CD207^{high} DC. B6 (**A, B**) or Lang-EGFP (**C**) mice were skin-painted with either DNFB (**A**) or DNTB (**A-C**) and, 72h later, DC were enriched from cutaneous LN and further sorted by flow cytometry into skin derived CD207$^+$ DC (CD8α$^-$EGFP^{high}), LN resident CD8α DC (CD8α$^+$EGFP$^{-/low}$) and DN DC ((CD8α$^-$EGFP$^-$), as shown in **Figure 3A**. For some experimental groups, the DNTB-painted ear skin was surgically excised 5 hours after hapten delivery (**B**). Various numbers of total DC (**A, B**) or isolated DC subsets (**C**) were cultured with DNP-specific CD8$^+$ effector T cells isolated from B6 mice at d+5 after DNFB immunization. Proliferation of CD8$^+$ T cells was determined after 3 days by Thymidine uptake and is expressed as mean cpm±SD of triplicate wells. Data are representative of 1 out of 2 (**B-C**) to 4 (**A**) independent experiments. *p< 0.05



GOMEZ DE AGUERO ET AL. (FIG. S3)

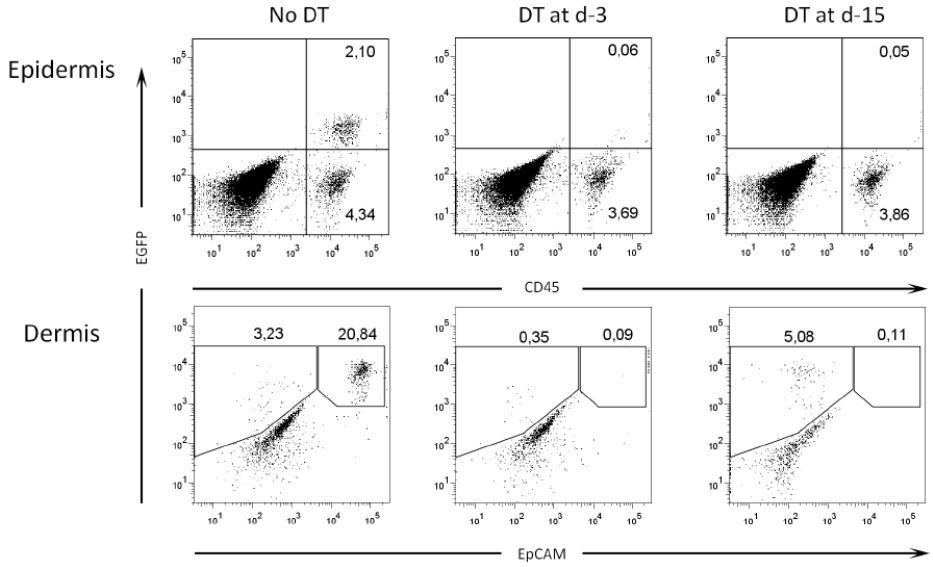
Supplemental Figure 3

All LN DC induce proliferation of specific CD8$^+$ T cells when pulsed *ex vivo* with Ag. Various DC subsets (**Figure 4A**) were purified from axillary and inguinal LN of Lang-EGFP mice, either naïve or 72h hours after abdominal skin painting with DNTB. They were then pulsed either with the SIINFEKL OVA peptide (**A**) or with DNBS (**B**), and 2.5x10^4 of each DC subsets were cultured with 1x10^5 OVA-specific CD8$^+$ T cells from OT-I transgenic mice (**A**) or DNP-specific CD8$^+$ effector T cells (**B**), respectively. Proliferation of CD8$^+$ T cells was determined after 3 days of culture by Thymidine uptake and is expressed as mean cpm±SD of triplicate wells. Data are representative of 1 out of 2 independent experiments.



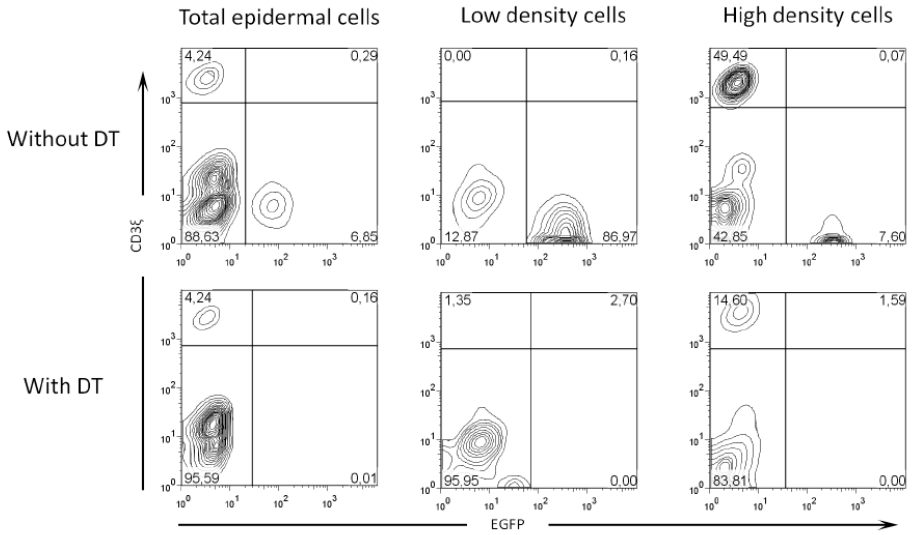
Supplemental Figure 4

In BM chimeric mice, presentation of DNTB in LN is mediated by host-derived CD207$^+$ LC but not donor-derived CD207$^+$ dDC. 3 types of Lang-EGFP BM chimeras were painted on the abdominal skin with DNTB and, 72 hours later, CD11c$^+$ cells were enriched from pooled inguinal and axillary LN and further separated into CD8$\alpha^{+}$$^{+}$EGFP$^{-/low}$ DC, CD8α^{-}EGFP^{-} DN DC and EGFP^{high} DC (left panels). Numbers indicate the percentage of each DC subset in gated CD11c$^+$ cells. LN EGFP^{high} DC from B6 ly5a (CD45.1)→Lang-EGFP(CD45.2) BM chimeras correspond to host-derived radioresistant LC and mostly contained CD103$^-$ cells, while those from Lang-EGFP→ly5a B6(CD45.1) BM chimeras represent donor-derived CD207$^+$ dDC and were mostly CD103$^+$ (middle panels). Serial dilutions of the DC subsets were cultured with DNP-specific CD8$^+$ T cells. The proliferative response was quantified by ^{3}H-TdR uptake after 3 days and is expressed as mean cpm±SD of triplicate wells. Data are representative of one out of two individual experiments. *p* values: *< 0.05



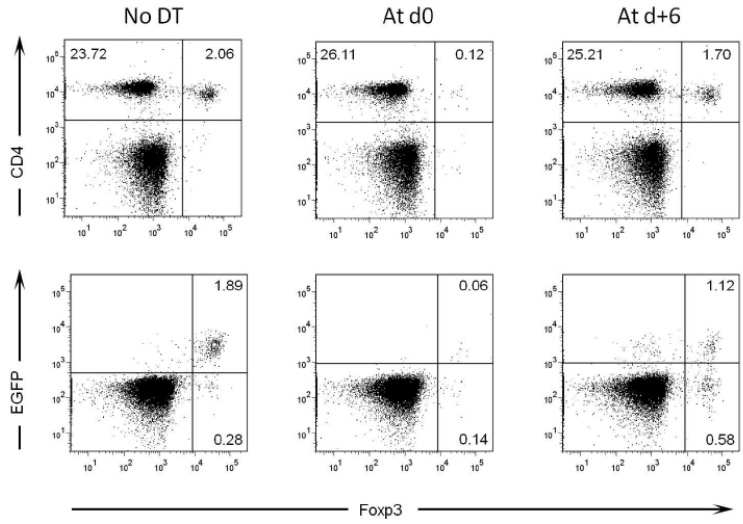
Supplemental Figure 5

Frequency of epidermal LC and CD207$^+$ dDC after DT injection at day-3 versus day-15. Epidermal and dermal cell suspensions were prepared from ears of Lang-DTR mice 3 or 15 days after DT injection and were analyzed by flow cytometry using anti-CD45, -CD11c and – EpCAM Abs. Total epidermal EGFP$^+$ cells correspond to LC, while EGFP$^+$ cells present in the dermis contain both CD207$^+$ dDC (EGFP$^+$EpCAM$^-$) and LC on their way to LN (EGFP$^+$ EpCAM $^+$). CD45 versus EGFP expression on total cells is shown for the epidermis (upper panels), while EpCAM versus EGFP expression on CD45$^+$CD11c$^+$ cells is shown for the dermis (lower panels). Whereas day-3 DT injected mice are devoid of the majority of LC and CD207$^+$ dDC, day-15 DT injected mice are deficient in LC in both epidermis and dermis, but harbor a normal proportion of CD207$^+$ dDC.



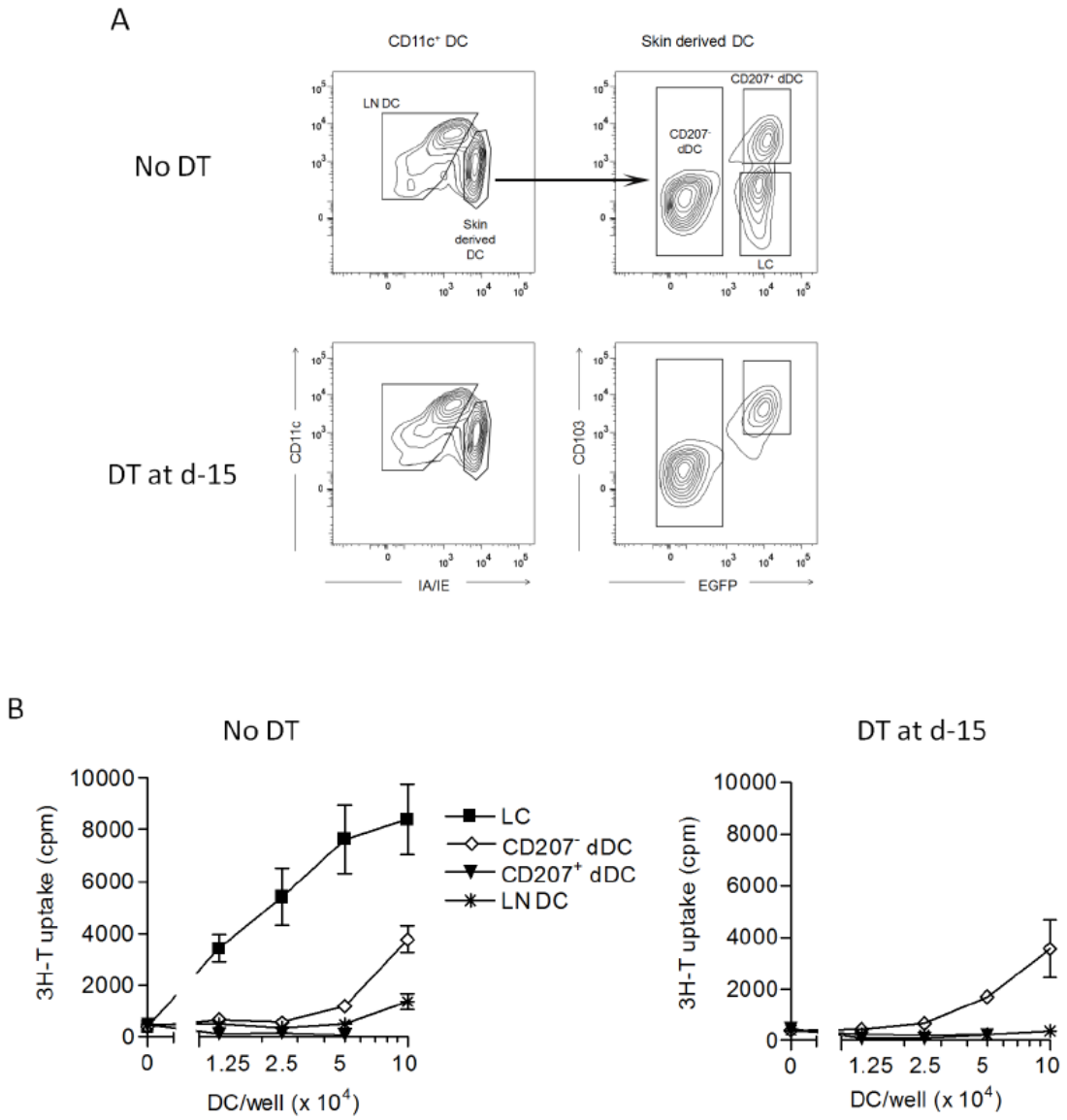
Supplemental Figure 6

Enrichment of epidermal LC and DETC by density gradient centrifugation. Lang-DTR mice were treated (lower panels) or not (top panels) with DT 3 days before DNTB ear painting. 4 hours later, ear epidermal cells were prepared by enzymatic digestion and centrifuged over an Optiprep® gradient. Flow cytometry analysis of EGFP (CD207) versus CD3ε expression revealed that the low density fraction contained 80-90% LC ($EGFP^+CD3\varepsilon^-$) and some keratinocytes (DN cells), while high density cells contained mostly DETC ($EGFP^-CD3\varepsilon^+$) and keratinocytes, but few LC. Low density cells prepared from DT-injected donor mice comprised almost exclusively keratinocytes and no LC. Similar purities were obtained when donor mice were painted with AOO as control.



Supplemental Figure 7

$CD4^+Foxp3^+$ Tregs recovered in DEREG mice within a week after DT injection. Foxp3-DTR/EGFP mice (DEREG) were injected with DT twice daily at days -2 and -1 and the frequency of $CD4^+Foxp3^+$ Tregs was determined in skin LN at day 0 (middle panel) and day 6 (right panel). Most $CD4^+Foxp3^+$ Tregs in non-injected DEREG mice were $EGFP^+$ and were efficiently depleted in LN at day 0. At day 6, $Foxp3^+$ Tregs have recovered to >80% of their normal frequency in LN and a significant proportion of them do not express EGFP.



Supplemental Figure 8

Dermal CD207$^-$ DC are the sole DC responsible for presentation of DNTB-modified peptides in LN of LC-depleted mice. At day-15 before abdominal skin painting with DNTB, Lang-DTR mice were injected with DT to selectively deplete LC. At day+3, LN resident DC (CD11c$^+$MHC-II^{int}), CD207$^-$ dDC (CD11c$^+$MHC-II^{high}EGFP$^-$CD103$^-$), CD207$^+$ dDC (CD11c$^+$MHC-II^{high}EGFP^{high}CD103$^+$) and LC (CD11c$^+$MHC-II^{high}EGFP^{high}CD103$^-$) were FACS sorted from pooled inguinal, axillary and brachial LN (**A**) and were cultured at serial dilutions with 10^5 DNP-specific CD8$^+$ effector T cells (**B**). Proliferation of CD8$^+$ T cells was determined after 3 days by Thymidine uptake and is expressed as mean cpm±SD of triplicate wells. Data are representative of 1 out of 2 independent experiments.